



BEFORE THE BOARD OF APPEALS AND INTERFERENCES  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Terry et al.

Serial No. 09/365,349

Filed: July 30, 1999

For: *Heavy Metal Phytoremediation*

Group Art Unit: 1638

Examiner: Ibrahim, M.

Attorney Docket No. B99-085

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Signed

Richard Osman

BRIEF ON APPEAL

The Commissioner of Patents  
Washington, D.C. 20231

Dear Commissioner:

This is an appeal from the 4/24/00 final rejection of claims 1-24.

REAL PARTY IN INTEREST

The real party in interest is the Regents of the University of California., the assignee of this patent application.

RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

STATUS OF THE CLAIMS

Claims 1-24 are pending and subject to this appeal.

STATUS OF THE AMENDMENTS

Claim 1 was amended after final to clarify that the term enhanced is relative; i.e. as

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compared with a corresponding a wild-type plant. The Examiner indicated this amendment would be entered. Accordingly, all Amendments are believed to be properly before the Board.

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### SUMMARY OF THE INVENTION

The claims are directed to a plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant. In particular embodiments, the plant comprises a gene encoding the glutamylcysteine synthetase operably linked to a heterologous promoter and is a member of the brassicaceae family, such as *Brassica juncea*. Applicable heavy metals include chromium, molybdenum, tungsten, cadmium, mercury and uranium. In more particular embodiments, the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant and/or the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions. See specification, p.3, lines 18-23 and pending claims.

The pending method claims provide for decreasing heavy metal content of a medium (such as soil), comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a subject plant in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased. See specification, p.3, lines 23-26 and pending claims.

### ISSUES

- I. WHETHER CLAIMS 1, 2, 5-8, 13-15 and 19 ARE PATENTABLE UNDER 35USC102(a).
- II. WHETHER CLAIMS 1-24 ARE PATENTABLE UNDER 35USC103(a).

### GROUPING OF THE CLAIMS

Claims 1-19 shall be considered together as a group and claims 20-24 shall be considered as a group.

ARGUMENT

## I. CLAIMS 1, 2, 5-8, 13-15 and 19 ARE PATENTABLE UNDER 35USC102(a).

The claims require a plant which is genetically engineered to overexpress glutamylcysteine synthetase *and thereby provides enhanced heavy metal accumulation*. The Action suggests that the poplars of Arisi et al. might inherently provide enhanced heavy metal accumulation. However, Arisi's subsequent report demonstrates that these poplars do not provide enhanced heavy metal accumulation, i.e. they showed no increased heavy metal accumulation compared with untransformed controls (Noctor et al., p.640, col.1, last paragraph - cited on p.3, line 9 of our Specification). As Arisi's poplars do not provide "enhanced heavy metal accumulation" they do not and can not meet or suggest the limitations of our claims.

There are innumerable possible reasons why Arisi's poplars fail to meet the limitations of the pending claims. Such speculation is idle, for the fact that they do fail to meet the limitations of the claims closes the 102(a) analysis.

## II. CLAIMS 1-24 ARE PATENTABLE UNDER 35USC103(a).

Raskin et al. "contemplates" (Raskin, column 4, line13) that plants engineered to express high levels of metallothioneins (MT's) could be used for phytoremediation of metals.<sup>1</sup> Watanabe et al. describes an E. coli gamma-glutamylcysteine synthetase (ECS) gene and Chen et al. (1994) report that mutant tomato cells selected for cadmium tolerance show increased ECS activity. The issue then, is whether the cited art suggests modifying Raskin to overexpress ECS, rather than an MT, and thereby secure a plant providing enhanced heavy metal accumulation.

A detailed reading of Chen and subsequent work from Chen's laboratory (not cited in the Action) reveals that the prior art not only fails to suggest the claimed invention, but in fact teaches directly away from it. In their discussion section, Chen acknowledges that the

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<sup>1</sup>The Final Action slightly overstates this reference. For example, Raskin does not "teach that Brassica juncea overexpressing metallothioneins accumulate heavy metals from metal contaminated soils". Raskin never suggests he made any such plant and never suggests that if he had made such a plant, it would have accumulated heavy metals. All Raskin offers is a suggestion (by prophetic Example 5) that Brassica juncea be transformed with a monkey MT gene and resultant transgenic lines be *evaluated* for heavy metal utilization. However, even as overstated, the citation (as metallothionein overexpression generally) has little relevance to the pending claims.

relationships between ECS activity, glutathione synthetase (GS) activity, phytochelatin (PC) synthesis, heavy metal tolerance and heavy metal accumulation are by no means clear. While Chen's results are similar to those of Steffens et al. (1989), cited by Chen on p.238 col 1, lines 50-53, other published reports suggest the opposite. For example, at p.238, col 2, line 20-25 Chen also cites de Knecht et al. (1992) for demonstrating that Cd-tolerant plants can synthesize fewer PCs than sensitive plants exposed to the same Cd concentration. Other data cited by Chen suggest that this mechanism of Cd-tolerance may not provide a practical route for generating useful plants. First, Chen's Cd-tolerance is not stable (Chen, p.238, col 1, lines 12-14) and second, such metal tolerant plants demonstrate poor growth characteristics (Chen, p.238, col 1, lines 22-25). Chen concludes by suggesting that future development of transgenic plants with altered capacities to synthesize either GSH or PCs might be used to test their hypothesis that increased GSH and/or PC synthesis increases Cd tolerance.

The senior author of Chen et al. subsequently reported on exactly these experiments (see our Specification, p.3, lines 5-9 and the Goldsbrough, 1999, reference cited therein) and like Arisi's poplars, Goldsbrough's transformed Arabidopsis plants provided no increase in heavy metal accumulation compared with controls. Specifically, Goldsbrough reports that while ECS could restore some degree of Cd tolerance to a Cd-sensitive mutant (a *cad2* mutant having reduced GSH levels), this gene did not increase Cd tolerance of wild type plants (Goldsbrough, p.230, line 35)<sup>2</sup>. Interestingly, Goldsbrough also further confounds the teachings of Chen by reporting that the ECS gene does not show any change in RNA expression in plants or cells that are exposed to Cd (Goldsbrough, p.230, lines 28-30).

The prior art does not suggest modifying Raskin to overexpress ECS, rather than an MT, and thereby secure a plant providing enhanced heavy metal accumulation. The prior art establishes an uncertain and unpredictable relationship between ECS expression and heavy metal

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<sup>2</sup> The final action suggests that the positive result with the Cd-sensitive mutants supports the rejection. We believe that would only be true if the claims encompassed plants which accumulate normal amounts of heavy metal. However, the present invention and pending claims do not relate to sensitive mutant plants restored by genetic engineering to accumulate normal amounts of heavy metal. The invention relates to hyper-accumulators. The claims now clarify that enhanced means enhanced over normal, wild-type accumulation - the claims do not and were never intended to encompass a Cd-sensitive mutant engineered to provide merely normal, wild-type heavy metal accumulation.

accumulation, and specifically teaches (in both Noctor et al. and Goldsbrough) that over expression of ECS will not yield heavy metal accumulators.

Absent a prior art suggestion to modify Raskin's metallothionein transformed plants to overexpress glutamylcysteine synthetase and thereby provide enhanced heavy metal accumulation - as required by all the pending claims - the claims are in compliance with 35USC103(a).

(a) Claims 20-24 further distinguish the cited art.

Claims 20-24 specifically require that the genetically engineered plant is otherwise phenotypically the same as a corresponding untransformed plant. This additional "normal growth" requirement is specifically discouraged by the prior art, see, e.g. Chen at p.238, col 1, lines 22-26 ("Metal-tolerant plants have also been shown to produce less biomass and have reduced fitness compared to their nontolerant counter-parts when grown in normal soil").

Applicants respectfully request reversal of the pending Final Action by the Board of Appeals. Applicants hereby petition for any necessary extension of time pursuant to 37 CFR 1.136(a). The Commissioner is hereby authorized to charge any necessary fees (small entity) or credit any overcharges associated with this communication to our Deposit Account No. 19-0750 (order no.B99-085).

Respectfully submitted,  
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## CLAIMS ON APPEAL

1. A plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant.
2. A plant according to claim 1 comprising a gene encoding the glutamylcysteine synthetase operably linked to a heterologous promoter.
3. A plant according to claim 1 which is a member of the brassicaceae family.
4. A plant according to claim 1 which is a *Brassica juncea*.
5. A plant according to claim 1 wherein the heavy metal is selected from the group consisting of chromium, molybdenum and tungsten.
6. A plant according to claim 1 wherein the heavy metal is selected from the group consisting of cadmium and mercury.
7. A plant according to claim 1 wherein the heavy metal is uranium.
8. A plant according to claim 1, wherein the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.
9. A plant according to claim 1, wherein the plant comprises a gene encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the plant is a *Brassica juncea*, the heavy metal is selected from the group consisting of chromium, molybdenum and tungsten and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.
10. A plant according to claim 1, wherein the plant comprises a gene encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the plant is a *Brassica*

*juncea*, the heavy metal is selected from the group consisting of cadmium and mercury and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.

11. A plant according to claim 1, wherein the plant comprises a gene encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the plant is a *Brassica juncea*, the heavy metal is selected from the group consisting of tellurium and polonium and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.

12. A plant according to claim 1, wherein the plant comprises a gene encoding the glutamylcysteine synthetase operably linked to a heterologous promoter, the plant is a *Brassica juncea*, the heavy metal is uranium and the enhanced accumulation is at least 50% greater than an otherwise comparable untransformed plant.

13. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 1 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

14. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 7 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

15. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 8 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy

metal, whereby the heavy metal content of the medium is decreased.

16. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 9 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

17. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 10 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

18. A method for decreasing heavy metal content of a medium, comprising the steps of: (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a plant according to claim 11 in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

19. A method according to claim 13, wherein the medium is soil.

20. A plant according to claim 1 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

21. A plant according to claim 4 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

22. A method according to claim 13 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.



23. A method according to claim 16 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

24. A method according to claim 17 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.